The term "polymerase chain reaction" refers to a method for amplifying a DNA base sequence using a heat-stable DNA polymerase and two oligonucleotide primers, one complementary to the (+)-strand at one end of the sequence to be amplified and the other complementary to the (-)-strand at the other end. Because the newly synthesized DNA strands can subsequently serve as additional templates for the same primer sequences, successive rounds of primer annealing, strand elongation, and dissociation produce rapid and highly specific amplification of the desired sequence. The polymerase chain reaction is used to detect the presence of polynucleotides encoding cytokines in the sample. Many polymerase chain methods are known to those of skill in the art and may be used in the method of the invention. For example, DNA can be subjected to 30 to 35 cycles of amplification in a thermocycler as follows: 95°C for 30 sec, 52° to 60°C for 1 min, and 72°C for 1 min, with a final extension step of 72°C for 5 min. For another example, DNA can be subjected to 35 polymerase chain reaction cycles in a thermocycler at a denaturing temperature of 95°C for 30 sec, followed by varying annealing temperatures ranging from 54-58°C for 1 min, an extension step at 70°C for 1 min and a final extension step at 70°C.

[0039] The primers for use in amplifying the polynucleotides of the invention may be prepared using any suitable method, such as conventional phosphotriester and phosphodiester methods or automated embodiments thereof so long as the primers are capable of hybridizing to the polynucleotides of interest. One method for synthesizing oligonucleotides on a modified solid support is described in U.S. Patent No. 4,458,066. The exact length of primer will depend on many factors, including temperature, buffer, and nucleotide composition. The primer must prime the synthesis of extension products in the presence of the inducing agent for amplification.

[0040] Primers used according to the method of the invention are complementary to each strand of nucleotide sequence to be amplified. The term "complementary" means that the primers must hybridize with their respective strands under conditions which allow the agent for polymerization to function. In other words, the primers that are complementary to the flanking sequences hybridize with the flanking sequences and permit amplification of the nucleotide sequence. Preferably, the 3' terminus of the primer that is extended has perfectly base paired complementarity with the complementary flanking strand.

[0041] Those of ordinary skill in the art will know of various amplification methodologies which can also be utilized to increase the copy number of target nucleic acid. The polynucleotides detected in the method of the invention can be further evaluated, detected, cloned, sequenced, and the like, either in solution or after binding to a solid support, by any method usually applied to the detection of a specific nucleic acid sequence such as another polymerase chain reaction, oligomer restriction (Saiki *et al., Bio/Technology 3*:1008-1012 (1985)), allele-specific oligonucleotide (ASO) probe analysis (Conner *et al., Proc. Natl. Acad. Sci. USA 80*: 278 (1983), oligonucleotide ligation assays (OLAs) (Landegren *et al., Science 241*:1077 (1988)), RNAse Protection Assay and the like. Molecular techniques for DNA analysis have been reviewed (Landegren *et al, Science 242*: 229-237 (1988)). Following DNA amplification, the reaction product may be detected by Southern blot analysis, without using radioactive probes. In such a process, for example, a small sample of DNA containing the polynucleotides obtained from the tissue or subject are amplified, and analyzed via a Southern blotting technique. The use of non-radioactive probes or labels is facilitated by the high level of the amplified signal. In one embodiment of the invention, one nucleoside triphosphate is radioactively labeled, thereby allowing direct visualization of the amplification product by autoradiography. In another embodiment, amplification primers are fluorescently labeled and run through an electrophoresis system. Visualization of amplified products is by laser detection followed by computer assisted graphic display, without a radioactive signal.

[0042] Simple visualization of a gel containing the separated products may be utilized to determine the presence or severity of a dermatitis reaction. For example, staining of a gel to visualize separated polynucleotides, a number of stains are well known to those skilled in the art. However, other methods known to those skilled in the art may also be used, for example scanning densitometry, computer aided scanning and quantitation as well as others.

[0043] Thus, the methods described above can be used to non-invasively obtain a sample of tissue from a subject suspected of having dermatitis, such as an irritant or allergic reaction, and isolate polynucleotides from the sample. The polynucleotides can then be analyzed using methods such as, but not limited to, those described above. Any number of cytokine levels can be quantified by measuring their relative expression in the sample obtained and comparing these

levels to normal-standard samples. For example, the mRNA level(s) in a cell change when the production of proteins in the skin are either increased or reduced. Thus, a measurement of RNA, in particular mRNA, provides a monitor of event(s) such as inflammatory reactions occurring in the skin or as a result of a local or systemic response. It will be recognized that the present non-invasive techniques are capable of detecting any reaction, disorder, or disease so long as the biological factor is present in the skin, more particularly below the stratum corneum of the skin. For example, and not by way of limitation, the inventors have discovered that polynucleotide encoding the cytokine IL-4 can be detected at higher levels in allergic contact dermatitis (ACD) lesions than in normal skin or skin from an ICD lesion. In addition, the inventors have discovered that polynucleotide encoding IL-13 is at a higher concentration in ACD skin than in normal or ICD skin. In contrast polynucleotide encoding IL-8 is at higher levels in both ACD and ICD compared to normal skin. Thus, elevated levels of IL-8 polynucleotide can be used diagnostically to detect a general contact dermatitis. By using the methods of the invention it is possible to quantify the severity of a reaction by measuring the levels of polynucleotides encoding cytokines when compared to a normal-standard sample.

[0044] The method for detecting a cytokine for distinguishing dermatitis reactions may alternatively employ the detection of a cytokine polypeptide. The method for detecting a cytokine polypeptide in cells is useful for distinguishing a reaction by measuring the level of a particular cytokine, for example IL-4, IL-8 and/or IL-13, in cells obtained from a subject suspected of having a dermatitis reaction. The levels of such cytokines are indicative of a reaction when compared to a normal or standard cytokine polypeptide profile in a similar tissue. Thus, the expression pattern of a cytokine polypeptide will vary depending upon the type and degree of a dermatitis reaction. In this regard, the sample obtained, as described herein, may be used as a source to isolate polypeptides. Measurement of a particular polypeptide, for example IL-4, may serve as a method of identifying ACD. For example, following skin scraping or skin stripping, using the methods described above, cells isolated from the stratum corneum may be lysed by any number of means, and polypeptides obtained from the cells. These polypeptides may then be quantified using methods known to those of skill in the art, for example by ELISA.